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REVIEW

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Semisynthetic aminoglycoside antibiotics: Development and enzymatic modifications

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Abstract
The critical resistance mechanisms of aminoglycoside antibiotics in bacteria of clinical importance are the enzymatic *N*-acetylation, *O*-phosphorylation, and *O*-nucleotidylation that generally result in the inactivation of aminoglycosides. To overcome such resistance mechanisms, dibekacin (3′,4′-dideoxykanamycin B) was developed as the first rationally designed semisynthetic aminoglycoside, based on the enzymatic 3′-*O*-phosphorylation of kanamycin. Subsequently, amikacin, netilmicin, and isepamicin were developed by introducing (*S*)-4-amino-2-hydroxybutyryl (AHB), ethyl, and (*S*)-3-amino-2-hydroxypropionyl side chains into the 1-amino group of kanamycin, sisomicin, and gentamicin B, respectively. These side chains are believed to block the access of a variety of aminoglycoside-modifying enzymes to their target sites. The latest semisynthetic aminoglycoside of clinical use in Japan is arbekacin (1-*N*-AHB-dibekacin), which has been extensively used since its approval as an anti-methicillin-resistant *Staphylococcus aureus* (MRSA) agent in 1990. Although it has several possible modification sites for aminoglycoside acetyltransferases (AACs), arbekacin-resistant MRSA strains that have emerged in the past 8 years have been those with a low or moderate level of resistance, due to a bifunctional enzyme, AAC(6′)/APH(2″), at low incidence. To overcome AAC(6′)/APH(2″)-dependent arbekacin-resistant MRSA strains, 2″-amino-2″-deoxyarbekacin and its 5-epiamino derivative have been already synthesized. However, simulative modification studies using AACs from aminoglycoside-producing *Streptomyces* strains have revealed that AAC(3) and AAC(2′) converted arbekacin to 3″-*N*-acetyl and 2′-*N*-acetyl derivatives, respectively, which retain high antibiotic activity. By contrast, the same acetylations of amikacin (3″-*N*-) and dibekacin (3-*N*-) resulted in their inactivation. Thus, these new findings confirmed the steric hindrance effect of the 1-*N*-acyl side chain and illuminated the novel aspect of arbekacin distinct from the other semisynthetic aminoglycosides, indicating that MRSA strains cannot be arbekacin-resistant even if they have acquired the *aac(3)* or *aac(2′)* gene.

Key words Aminoglycoside antibiotics · Resistance mechanism · Aminoglycoside-modifying enzymes · Semisynthetic · Arbekacin · *Streptomyces* origin

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Introduction

The first aminoglycoside antibiotic, streptomycin,[1] was discovered by Waksman in 1944, and has been widely used for the treatment of bacterial infections, in particular, tuberculosis. A few years later, another aminoglycoside antibiotic was found independently by Umezawa et al.[2,3] who termed it streptothricin B (fradiomycin) and by Waksman and Lechevalier who termed it neomycin.[4] The clinical use of penicillin, streptomycin, chloramphenicol, and tetracycline resulted in the emergence of drug-resistant bacteria, and staphylococci and gram-negative bacteria resistant to all these antibiotic agents caused serious infections. Kanamycin (produced by *Streptomyces kanamyceticus*), discovered by Umezawa et al.[5] in 1957, has been used clinically as an effective agent for the treatment of infections with these drug-resistant bacteria, including streptomycin-resistant tuberculosis. However, as a result of its widespread use, in 1965 kanamycin-resistant strains appeared in patients, at a low incidence. Umezawa et al.[6–11] then began studies of the biochemical mechanisms of resistance to aminoglycoside antibiotics, and in 1967 the enzymatic mechanisms were first elucidated, demonstrating three types of aminoglycoside-modifying enzymes in clinically isolated resistant bacteria carrying R plasmids. These are aminoglycoside acetyltransferases (AAC), phosphotransferases (APH), and adenylyltransferases (AAD), which

modify the amino and hydroxyl groups at specific sites in the antibiotics. On the basis of these findings, Umezawa et al. predicted structures refractory to these enzymes, and synthesized many aminoglycoside derivatives in order to develop agents that would be effective against resistant bacteria. In 1975, dibekacin was selected for chemotherapeutic use as the first rationally designed semisynthetic aminoglycoside.

In this article, we discuss the enzymatic mechanisms of resistance to aminoglycoside antibiotics, and the semisynthetic modifications of antibiotics including the development of arbekacin, which is effective against methicillin-resistant *Staphylococcus aureus* (MRSA). The enzymatic modifications of arbekacin by new AACs of *Streptomyces* origin are also described.

Useful aminoglycoside antibiotics

In general, the term "aminoglycoside antibiotics (or aminoglycosides)" refers to a group of antibiotics possessing a glycosidic linkage(s) with aminosugar(s) or pseudo-aminosugar (aminocyclitol). Basic glycosides containing aglycones, such as the macrolide, nucleoside, and anthracycline antibiotics, are not included. The stereostructures of all aminoglycoside antibiotics described in this review are based on absolute structures confirmed by X-ray crystallographic studies on kanamycin, streptomycin, and other antibiotics.

Most aminoglycoside antibiotics exhibit a broad antimicrobial spectrum and strongly bactericidal activity against mycobacteria, staphylococci, and gram-negative bacteria, including pseudomonads, but have little effect in inhibiting the growth of streptococci, pneumococci, and anaerobic bacteria. More than 150 naturally occurring aminoglycosides have been isolated from culture broths of actinomycete and bacterial strains.[11–13] Most aminoglycoside antibiotics important for chemotherapy contain a 1,3- or 1,4-diaminocyclitol named streptidine, actinamine, 2-deoxystreptamine, or fortamine (Table 1 and Fig. 1). Among these, streptomycin, spectinomycin, neomycins, paromomycins, ribostamycin, kanamycin, bekanamycin (kanamycin B), tobramycin, gentamicins, sisomicin, micronomicin, and astromicin (fortimicin A) are available as chemotherapeutic agents. Five semisynthetic aminoglycosides; dibekacin and arbekacin (derived from bekanamycin), amikacin (derived from kanamycin), netilmicin (derived from sisomicin), and isepamicin (derived from gentamicin B) are marketed as chemotherapeutic agents active against resistant bacteria. Hygromycin B and destomycin A are used as animal anthelmintics. Kasugamycin, validamycin A, and some aminoglycosides are used for the prevention of plant diseases. Geneticin (G-418) and a few other aminoglycosides are available as biochemical reagents. The structures of clinically used kanamycin and gentamicin antibiotics are shown in Fig. 2.

Enzymatic mechanisms of resistance in clinical isolates

The most important mechanism of resistance to aminoglycoside antibiotics among resistant bacteria of clinical origin arises from enzymatic *N*-acetylation, *O*-phosphorylation, and *O*-nucleotidylation of specific sites in the antibiotics. The genes for these aminoglycoside-modifying enzymes are located mainly on plasmids. Organisms with resistance due to permeability barriers to agents, have

Table 1. Useful aminoglycoside antibiotics containing 1,3- or 1,4-diaminocyclitols

Diaminocyclitol	Glycosidic substitution	Antibiotic group	Naturally-occurring	Semisynthetic
Streptidine	4-	Streptomycin	Streptomycin[a]	
Actinamine	4,5-	Spectinomycin	Spectinomycin[a]	
2-Deoxystreptamine	4-	Apramycin	Apramycin	
	5-	Destomycin	Destomycin A[b]	
			Hygromycin B[b]	
	4,5-	Neomycin	Neomycins[a]	
			Paromomycins[a]	
			Lividomycins	
			Ribostamycin[a]	
			Butirosins	
	4,6-	Kanamycin	Kanamycin[a]	Dibekacin[a]
			Bekanamycin[a]	Amikacin[a]
			Tobramycin[a]	Arbekacin[a]
	4,6-	Gentamicin	Gentamicin C[a]	Netilmicin[a]
			Gentamicin B	Isepamicin[a]
			Micronomicin[a]	
			Sisomicin[a]	
			Geneticin[c]	
Fortamine	6-	Fortimicin	Astromicin[a]	

[a] Clinical chemotherapeutic.
[b] Veterinary anthelmintic agent.
[c] Biochemical reagent.

also been isolated. But ribosomal resistance to aminoglycosides is very rare in clinically isolated bacteria. These enzymatic mechanisms have been reviewed by Umezawa,[8,9] Davies and Smith,[14] and Umezawa and Kondo.[10,11] Many genes encoding aminoglycoside-modifying enzymes were appropriately reviewed by Shaw et al.[15] The nomenclature and abbreviations for these aminoglycoside-modifying enzymes and resistant genes were proposed by Mitsuhashi.[16]

In 1965 Okamoto and Suzuki[17] reported that an intracellular enzyme in *Escherichia coli* K12 R5 transferred the acetyl group of acetyl coenzyme A (CoA) to chloramphenicol. The strain was obtained by transmission of an R plasmid from a natural isolate of multiple drug-resistant dysentery bacteria. In 1967, the Umezawa group[6,18] isolated the reaction product of kanamycin with the homogenate of this strain, and determined the structure to be 6′-*N*-acetylkanamycin. Following this, Mitsuhashi obtained *Eschericia coli* K12 ML1629, which was highly resistant to all kanamycins and neomycins, by transmission of an R plasmid from a clinically isolated resistant strain of *E. coli* to nalidixic acid-resistant *E. coli* K12 ML1410. The Umezawa group[7,19,20] demonstrated that the homogenate of the strain catalyzed the transfer of the terminal phosphate group of ATP to the 3′-hydroxyl of kanamycin. In 1968 they also first found an enzymatic modification of streptomycin by this strain, and the structure of the reaction product was determined to be streptomycin 3″-adenylate.[21,22] Independently, Yamada et al.[23] reported the inactivation of streptomycin by the adenylyltansferase in *E. coli* JE254.

Thus, the method of clarifying the enzymatic mechanisms of resistance to aminoglycoside antibiotics by structural elucidation of the enzymatic reaction products (which were purified by ion-exchange chromatography)[24] was established by the Umezawa group.[10,25] Besides these three aminoglycoside-modifying enzymes – AAC(6′), APH(3′), and AAD(3″) – a large number of other AACs, APHs, and AADs have been found in resistant strains by many researchers, as shown in Table 2. It is interesting that neither an acetyltransferase that modifies streptomycin nor a single enzyme that inactivates both streptomycin and kanamycin have been found. Recently, structures of enzymatic reaction products have been elucidated solely by spectrometric methods, including ^{1}H nuclear magnetic resonance (NMR), ^{13}C NMR, and mass spectrometric analyses.[10] In particular, the progress of two-dimensional NMR experiments has contributed to the accurate and rapid determination of the structure. Enzymatic modifications of kanamycin and bekanamycin by resistant bacteria are summarized in Fig. 3.

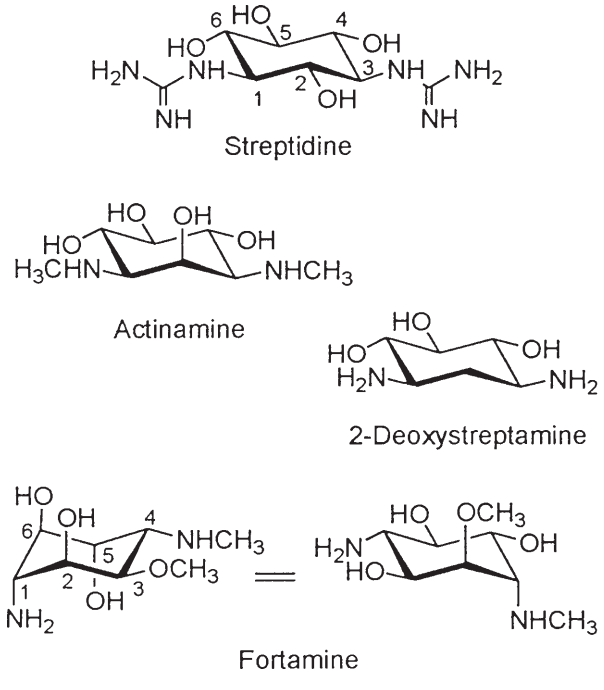

Fig. 1. Diamnocyclitols

Chemical modifications based on resistance mechanism

On the basis of enzymatic mechanisms of resistance to aminoglycoside antibiotics, the Umezawa group[8,10,13,26] initiated synthetic studies of derivatives that do not undergo the enzymatic reactions and inhibit the growth of resistant strains. The first agent synthesized based on the finding of the resistant mechanism, 6′,3″-di-*N*-methylkanamycin, inhibited the growth of *E. coli* producing AAC(6′), but showed weaker antibacterial activity against kanamycin-sensitive strains than the parent antibiotic.[27] Although 3′-*O*-methylkanamycin had only weak activity,[28] 3′-deoxykanamycin, which was synthesized by a complicated glycosidation method, showed excellent activity against both gram-positive and gram-negative bacteria, including resistant strains due to APH(3′).[29] This provided complete proof of the enzymatic mechanisms of resistance. Subsequently, 3′,4′-dideoxykanamycin B (dibekacin) was prepared, starting from kanamycin B (bekanamycin), and showed strong activity not only against resistant staphylococci and gram-negative bacteria, but also against *Pseudomonas*.[30] Dibekacin has been used in Japan since 1975 and is a useful chemotherapeutic agent. The successful result boosted the syntheses of numerous 3′-deoxy and 3′,4′-dideoxy derivatives active against resistant bacteria having APH(3′).

Another approach to semisynthetic aminoglycosides active against resistant bacteria is the acylation or alkylation of the 1-amino group in 2-deoxystreptamine-containing aminoglycosides. Kawaguchi et al.[31] first synthesized amikacin by the 1-*N*-acylation of kanamycin with (*S*)-4-amino-2-hydroxybutyric acid (AHB). The amino acid is contained in butirosins, aminoglycoside antibiotics produced by *Bacillus circulans*, which have shown activity against a variety of resistant bacteria.[32] Amikacin inhibits the growth of resistant bacteria having APH(3′)-I and AAD(2″) and has been used since 1977 for treating infections caused by resistant bacteria. Netilmicin (1-*N*-ethylsisomicin),[33] which has good activity against sensitive and resistant bacteria, has been marketed as a chemotherapeutic agent since 1985. Isepamicin[34] which was synthesized

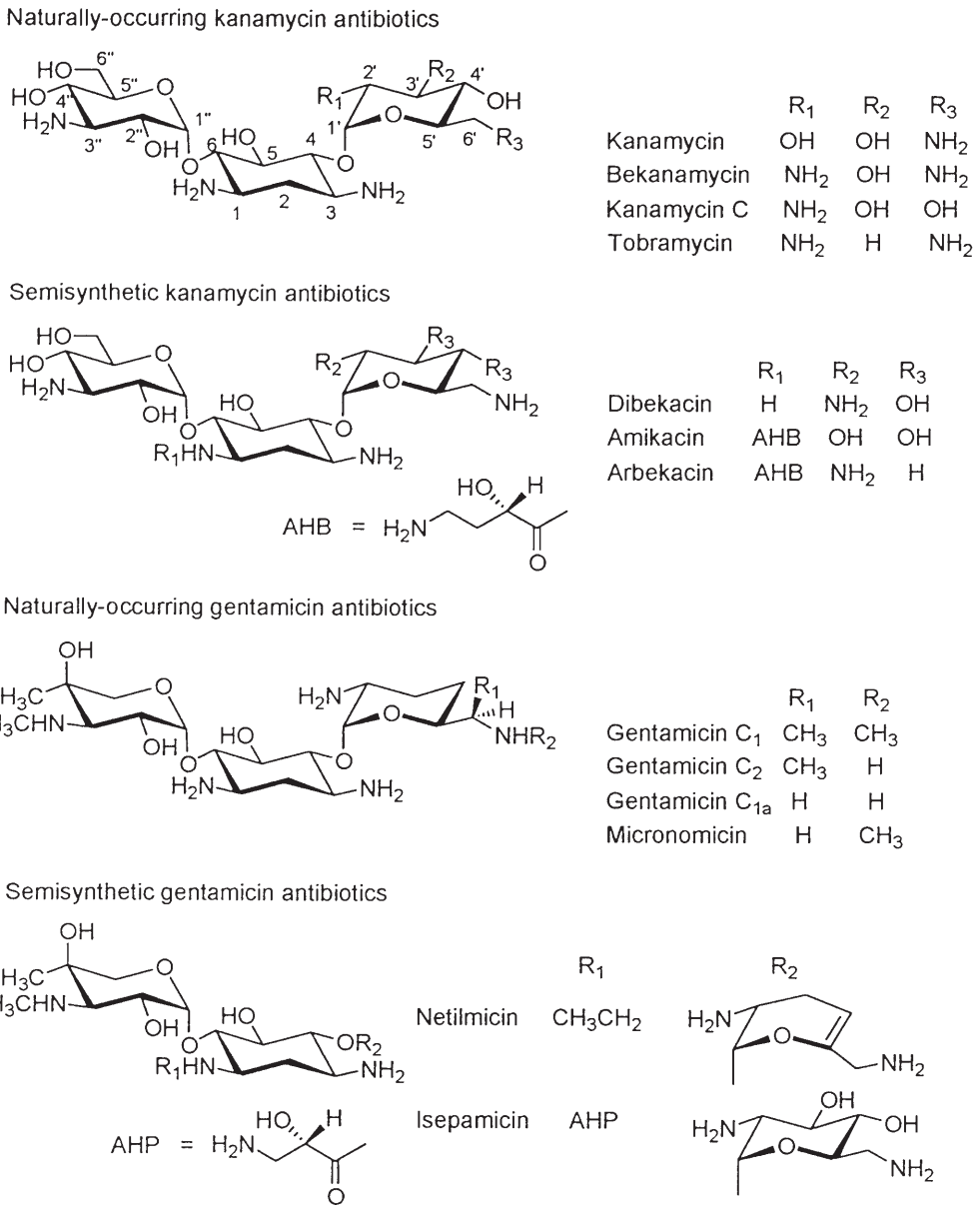

Fig. 2. 4,6-Disubstituted 2-deoxystreptamine aminoglycosides

by the 1-*N*-acylation of gentamicin B with (*S*)-3-amino-2-hydroxypropionic acid and launched in 1988.

The combination of deoxygenation and 1-*N*-acylation has provided further effective derivatives. Arbekacin, synthesized by the 1-*N*-acylation of dibekacin with AHB, showed strong activity against resistant bacteria, including *Pseudomonas*.[35] Accordingly, many deoxygenated derivatives of aminoglycoside antibiotics were synthesized and screened in efforts to develop new chemotherapeutic agents.[13] Polydeoxy derivatives, 5,2′,3′,4′,4″,6″-hexadeoxykanamycin and 5,3′,4′,4″,6″-pentadeoxykanamycin B, which have only one hydroxyl group at the 2″-position, were also active against gram-positive and gram-negative bacteria, except for *Pseudomonas* and some resistant bacteria producing AAC(6′) and AAD(2″). But 5,2′,3′,4′,2″,4″,6″-heptadeoxykanamycin, which had no hydroxyl group, had very weak activity. The AHB derivatives of hexadeoxykanamycin and pentadeoxykanamycin B showed strong activities.[36,37] It was concluded that the amino groups of kanamycin antibiotics play a critical role in antibacterial activity, and the 2″-hydroxyl and the AHB moiety on the 1-amino group markedly augments this activity. It has now become possible to prepare semisynthetic aminoglycosides which will be effective against resistant strains producing aminoglycoside-modifying enzymes which may appear in the future.

Development of arbekacin an agent effective against MRSA

In 1973, Kondo et al.[35,38] synthesized arbekacin, starting from dibekacin, by the acylation of the 1-amino group with AHB. Arbekacin[39–41] was refractory to most aminoglycoside-modifying enzymes in resistant bacteria

Table 2. Typical aminoglycoside-modifying enzymes in resistant bacteria of clinical isolates

Enzyme	Phenotype (modifying position)	Bacterial strain
AAC(3)-I	GMr, ASTMr(1-NH_2)	*Pseudomonas aeruginosa*, *Escherichia coli*, *Enterobacter*
AAC(3)-II	GMr, KMr	*Klebsiella*
AAC(3)-III	GMr, KMr, PRMr	*P. aeruginosa*
AAC(3)-IV	GMr, KMr, PRMr, APr	*E. coli*
AAC(2′)	GMr, TOBr, KMs	*Providencia*
AAC(6′)-I	KMr	*E. coli, Shigella*
AAC(6′)-II	KMr, GMr	*Moraxella*
AAC(6′)-III	KMr, GMr, DKBr	*P. aeruginosa*
AAC(6′)-IV	KMr, GMr, DKBr, AMKr	*P. aeruginosa*
AAC(6′)/APH(2″)	KMr, GMr, TOBr, ABKs	*Staphylococcus aureus* (MRSA)
APH(3′)-I	KMr, RSMr, LVr(5″-OH), GMs	*E. coli*, *P. aeruginosa*, *S. aureus*
APH(3′)-II	KMr, RSMr, BTr, GMs	*E. coli, P. aeruginosa*
APH(3′)-III	KMr, RSMr, BTr, LVr(5″-OH)	*P. aeruginosa*
APH(5″)	RSMr	*P. aeruginosa*
APH(6)	SMr	*P. aeruginosa*
APH(3″)	SMr	*E. coli, P. aeruginosa*, *S. aureus*
AAD(4′,4″)	KMr, TOBr, AMKr, DKBr(4″-OH)	*S. epidermidis*, *S. aureus*
AAD(2″)	KMr, GMr, AMKs	*E. coli*
AAD(6)	SMr	*S. aureus*
AAD(3″)	SMr, SPCMr(9-OH)	*E. coli*

AAC, aminoglycoside acetyltransferase; APH, aminoglycoside phosphotransferase; AAD, aminoglycoside adenylyltransferase; GM, gentamicin; ASTM, astromicin; KM, kanamycin; PRM, paromomycin; AP, apramycin; TOB, tobramycin; DKB, dibekacin; AMK, amikacin; ABK, arbekacin; RSM, ribostamycin; LV, lividomycin; BT, butirosin; SM, streptomycin; SPCM, spectinomycin; r, resitant; s, sensitive.

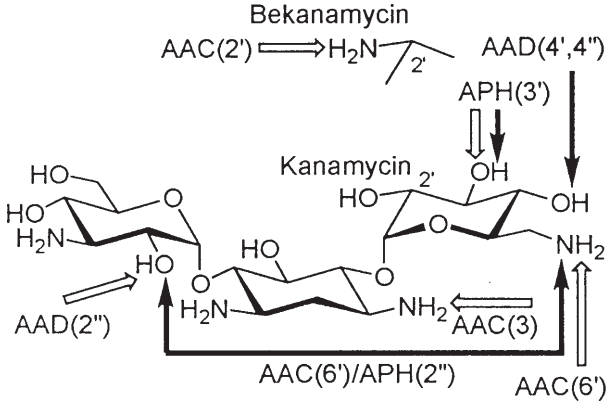

Fig. 3. Enzymatic modifications of kanamycin (2′-OH) and bekanamycin (2′-NH_2) by resistant bacteria. *Hollow arrows*, By gram-negative bacteria; *black arrows*, by methicillin-resistant *Staphylococcus aureus* (MRSA). *AAC*, Aminoglycoside acetyltransferase; *AAD*, Aminoglycoside adenylyltransferase; *APH*, Aminoglycoside phosphotransferase

(Fig. 4) and inhibited not only gram-negative bacteria, including *Pseudomonas*, but also staphylococci. An increase in the industrial yield to more than 70% led to clinical studies in the 1980s. Excellent effects and a low incidence of adverse reactions were confirmed in various clinical fields.[39] In 1984, Ubukata et al.[42] found that arbekacin was stable to aminoglycoside-modifying enzymes such as APH(3′), AAD(4′,4″),[43,44] and AAC(6′)/APH(2″)[45,46] in multiple drug-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*. The clinical efficacy of arbekacin against MRSA infections was confirmed, as expected.[47] Therefore, Professor Konno suggested developing arbekacin as an anti-MRSA agent for emergency use. Arbekacin has been approved for the specific treatment of MRSA infections since 1990 in Japan, and is still extensively used for this indication.

Arbekacin derivatives stable to enzymatic modification by MRSA

A small number of MRSA strains with a moderate level (minimum inhibitory concentration [MIC]; 6.25-25 μg/ml) of arbekacin resistance have been clinically isolated, but no highly resistant strains have been isolated. When arbekacin was modified by an in-vitro reaction using an excess amount of a crude enzyme preparation extracted from an arbekacin-resistant MRSA strain (12.5 μg/ml), three inactivated products, consisting mainly of arbekacin 2″-phosphate, along with small amounts of 6′-*N*-acetylarbekacin and doubly modified arbekacin, were isolated by Kondo et al. in 1993.[48,49] It was confirmed that the arbekacin resistance of clinically isolated MRSA strains was due mainly to a bifunctional enzyme, AAC(6′)/APH(2″), which has the capacity for both 2″-*O*-phosphorylation and 6′-*N*-acetylation in arbekacin. Very recently, Fujimura et al.[50] reported the acetylation of the 4‴-amino group of arbekacin in an in-vitro reaction using a crude enzyme preparation from an MRSA strain with low arbekacin resistance. According to their discussion, this acetylation was due to AAC(4‴) derived from AAC(6′)/APH(2″).[51]

Replacement of the 2″-hydroxyl group by an amino group in dibekacin or in arbekacin was designed by Kondo[40] to obtain potent active derivatives against the MRSA with

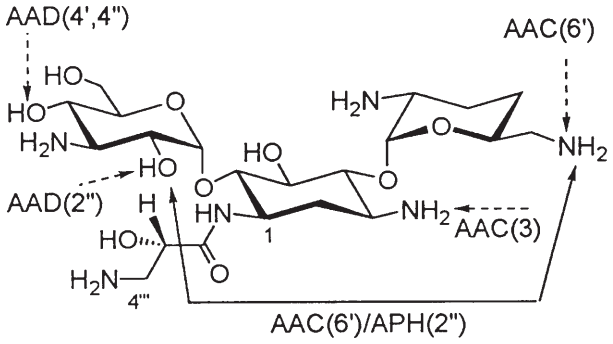

Fig. 4. Enzymatic modification of arbekacin by resistant bacteria. *Solid arrows*, by MRSA; *dotted arrows*, by gram-negative bacteria, to which arbekacin is refractive

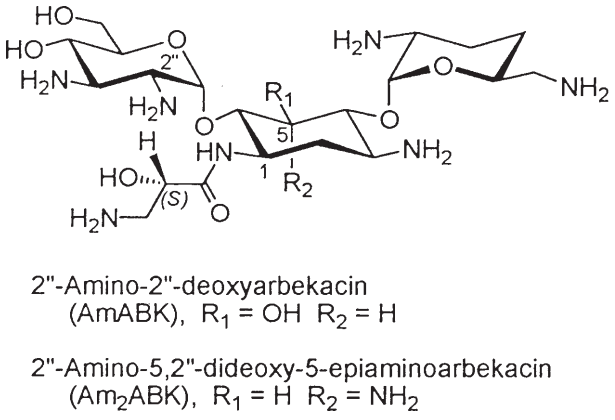

Fig. 5. Derivatives of arbekacin

the bifunctional enzyme. Conversion of the 2″-hydroxyl group by selective oxidation followed by reductive amination gave 2″-amino-2″-deoxydibekacin and 2″-amino-2″-deoxyarbekacin.[52] Their 5-deoxy, 5-epifluoro and 5-epiamino derivatives were also synthesized.[53] As expected, all 2″-amino-2″-deoxyarbekacin derivatives showed excellent activities against MRSA as well as against gram-negative bacteria. Among the derivatives 2″-amino-2″-deoxyarbekacin (AmABK) and its 5-epiamino derivative (Am_2ABK) (Fig. 5) were selected for further evaluation. Two derivatives showed in-vivo activity which paralleled the in-vitro MICs (Table 3), and were less toxic than arbekacin in terms of acute toxicity in mice (Table 4) and nephrotoxicity in rats.[54]

Modifications of arbekacin by new acetyltransferases of *Streptomyces* origin

According to a recent survey of clinical aminoglycoside resistance,[55] AACs were shown to be the most frequently occurring resistance factors. In this regard, arbekacin has modification sites for AAC(3), AAC(2′), and AAC(6′). Although MRSA strains with these AACs have not been reported thus far, one cannot rule out the possibility that such MRSA strains will emerge in the future. To check the possibility, Hotta et al.[56] attempted to use AACs available from *Streptomyces*, such as aminoglycoside producers. First, arbekacin and dibekacin were exposed to AAC(2′) derived from a kasugamycin-producing strain, *Streptomyces kasugaensis* MB273. Subsequently arbekacin was readily converted to 2′-*N*-acetylarbekacin, which retained antibiotic activity (42% of the activity of arbekacin against *Bacillus subtilis* PCI219 by an ordinary cup assay method), indicating that AAC(2′)-dependent aminoglycoside-resistant bacteria were not resistant to arbekacin.[56] By contrast, 2′-*N*-acetyldibekacin showed almost no activity. A small amount of the 2′,6′-di-*N*-acetyl derivatives of arbekacin and dibekacin were also formed by this enzymatic reaction. If these derivatives are produced by a single enzyme, then the AAC(2′) of *Streptomyces* origin should be a novel one (Fig. 6).

A new enzyme, AAC(3)-X, which acetylates the 3-amino group of kanamycin, was prepared from *S. griseus* SS-1198PR, (a kanamycin-resistant mutant derived from a wild type streptomycin-producing strain [*S. griseus* SS-1198]) by Hotta et al.[57] Subsequently, arbekacin, amikacin, and dibekacin were exposed to AAC(3)-X. Interestingly, arbekacin and amikacin, which have the 1-*N*-acyl side chain, were modified by acetylation at the 3″-amino group, which has never been reported in any aminoglycosides (Fig. 6), whereas dibekacin, which lacks the AHB side chain, was converted to the 3-*N*-acetyl derivative, as in the case of kanamycin.[58] On the other hand, two known enzymes, AAC(3)-III and AAC(3)-IV, produced by *Pseudomonas aeruginosa* PST1[59] and *Escherichia coli* JR225,[60] respectively, did not acetylate arbekacin and amikacin, but readily converted kanamycin and dibekacin to the 3-*N*-acetyl derivatives. A new product, 3″-*N*-acetylarbekacin, showed substantial antibiotic activity (55% of the activity of arbekacin against *Bacillus subtilis* PCI219), as in the case of 2′-*N*-acetylarbekacin.[56] By contrast, 3″-*N*-acetylamikacin and 3-*N*-acetyldibekacin showed 3% and 0.2% of the activities, respectively, of their parent antibiotics. Thus, the high antibiotic activities of these monoacetylated arbekacin derivatives represent a striking aspect of arbekacin distinct from the other aminoglycoside antibiotics. Arbekacin may be regarded as representing a new generation of aminoglycoside antibiotics, as shown in Fig. 7.

The 3″-*N*-acetylation should reflect a steric hindrance effect of the acyl side chain common to both arbekacin and amikacin. The 3″-*N*-acetylation will take place on the opposite side of the 3-amino group, possibly due to the effect of the side chain. The long arm of the panthoteine residue in the acetyl CoA molecule may also be critical for the 3″-*N*-acetylation. It should also be noted that AACs derived from *P. aeruginosa* and *E. coli* failed to produce the 3″-*N*-acetylation, although these enzymes produced 3-*N*-acetyl derivatives from kanamycin and dibekacin. This means that AAC(3)-X of *Streptomyces* origin has a unique catalytic property.

Table 3. Antimicrobial spectra of arbekacin and its 2″-amino derivatives

Test organism	Aminoglycoside-modifying enzyme	MIC (μg/ml)		
		ABK	AmABK	Am_2ABK
Staphylococcus aureus 209P		0.20	0.39	0.20
S. aureus Smith		≤0.10	≤0.10	≤0.10
S. aureus Ap01	AAD(4′,4″)	0.78	1.56	1.56
S. aureus MS16502 (MRSA)	AAC(6′)/APH(2″)	6.25	1.56	1.56
S. aureus MS16526 (MRSA)	AAC(6′)/APH(2″)	12.5	1.56	0.78
S. epidermidis 109	AAD(4′,4″)	0.78	1.56	0.78
Bacillus subtilis PCI219		≤0.10	0.20	0.20
Corynebacterium bovis 1810		0.39	0.78	3.13
Escherichia coli NIHJ		0.39	0.39	0.78
E. coli K-12		0.20	0.78	0.78
E. coli K-12 R5	AAC(6′)-1	12.5	12.5	50
E. coli K-12 J5 R11–2	APH(3′)-I	0.20	0.39	0.78
E. coli K-12 ML 1629	APH(3′)-I	0.78	1.56	3.13
E. coli K-12 ML 1410		0.78	3.13	1.56
E. coli K-12 ML 1410 R81	APH(3′)-I	0.78	1.56	1.56
E. coli K-12 LA290 R55	AAD(2″)	1.56	1.56	1.56
E. coli K-12 C600 R135	AAC(3)-I	0.39	1.56	3.13
E. coli W677		0.20	0.78	0.78
E. coli JR66/W677	APH(3′)-II, AAD(2″)	1.56	3.13	3.13
E. coli JR225	AAC(3)-IV	0.39	0.78	0.78
Klebsiella pneumoniae PCI602		0.78	1.56	0.78
K. pneumoniae 22#3038	APH(3′)-II, AAD(2″)	1.56	3.13	1.56
Shigella dysenteriae JS11910		1.56	3.13	3.13
Salmonella typhi T-63		0.78	0.78	0.78
S. enteritidis 1891		1.56	6.25	3.13
Proteus vulgaris OX19		0.78	1.56	1.56
Providencia sp. Pv16	AAC(2′)	1.56	1.56	0.78
Providencia sp. 2991	AAC(2′)	6.25	6.25	0.78
Serratia marcescens		6.25	6.25	3.13
Pseudomonas aeruginosa A3		≤0.10	0.78	0.78
P. aeruginosa No. 12		3.13	6.25	3.13
P. aeruginosa H9	APH(3′)-II	3.13	6.25	6.25
P. aeruginosa TI-13	APH(3′)-I	3.13	3.13	1.56
P. aeruginosa GN315	AAC(6′)-4	6.25	12.5	25
P. aeruginosa 99	AAC(3)-I	6.25	12.5	6.25
P. aeruginosa B-13	APH(3′)-I, -II	6.25	12.5	6.25
P. aeruginosa 21-75	APH(3′)-III	25	50	12.5
P. aeruginosa PST1	AAC(3)-III	6.25	12.5	3.13

ABK, Arbekacin; AmABK, 2″-amino-2″-deoxyarbekacin; Am_2ABK, 2″-amino-5,2″-dideoxy-5-epiaminoarbekacin; MIC, minimum inhibitory concentration (in vitro).

Table 4. In-vivo antibacterial activity and intravenous acute toxicity of arbekacin and its 2″-amino derivatives

Antibiotic	*S. aureus* MS16526 (MRSA)			*P. aeruginosa* GN10362		Acute toxicity
	MIC (μg/ml)	ED_{50}[a] (mg/mouse)	ED_{50}[b] (mg/mouse)	MIC (μg/ml)	ED_{50}[c] (mg/mouse)	LD_{50}[d] (mg/kg)
ABK	12.5	0.25	0.75	3.13	0.42	118
AmABK	1.56	0.33				>150
Am_2ABK	0.78		0.17	6.25	0.44	168

ED_{50}, Effective dose for 50% of group; LD_{50}, lethal dose for 50% of group.
[a] Eight ICR-Jcl male mice were used in each group, and antibiotics were administered intravenously. Challenge dose, 1.7×10^5 CFU/mouse (ip).
[b] Challenge dose, 7.1×10^5 CFU/mouse (ip).
[c] Challenge dose, 4.9×10^4 CFU/mouse (ip).
[d] Antibiotics were injected intravenously into ICR-Jcl male mice (five in each group).

The other point to note is that 3″-*N*-acetylarbekacin showed substantial antibiotic activity, whereas no significant activity was observed with 3″-*N*-acetylamikacin, despite their structural similarity. The differences can be seen at the 2′-, 3′- and 4′-positions. It seems possible that the presence of an extra amino group in arbekacin in comparison with amikacin plays a critical role in the antibiotic activity. However, this explanation cannot be acceptable for the substantial activity of 2′-*N*-acetylarbekacin compared with the activity of 2′-*N*-acetyldibekacin, as there is no difference in the numbers of free amino groups between them (Fig. 6).[56] Therefore, the reason for the antibiotic activity of the monoacetyl derivatives of arbekacin remains to be elucidated.

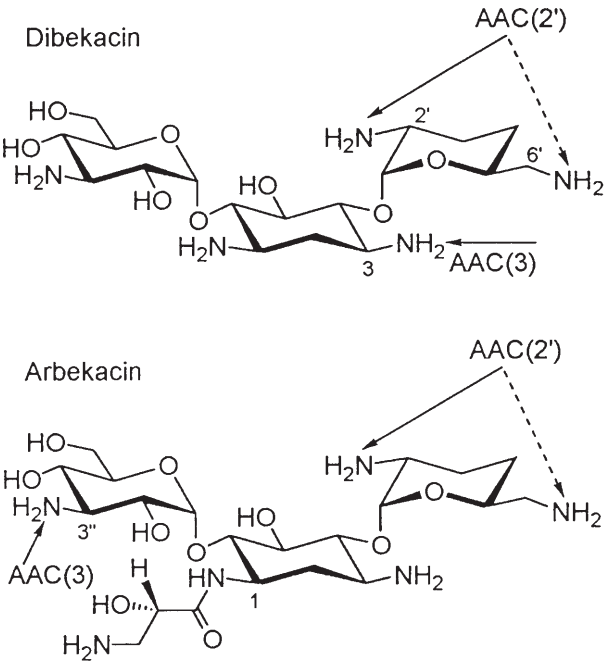

Fig. 6. Modification of dibekacin and arbekacin by aminoglycoside-modifying enzymes of *Streptomyces* origin

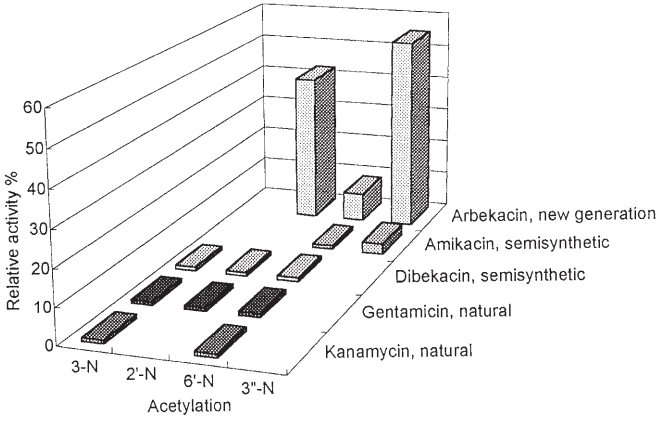

Fig. 7. Relative activities of monoacetylated aminoglycoside antibiotics

Concluding remarks

Refractoriness to aminoglycoside-modifying enzymes of clinical origin has been the key stimulus for the development of new semisynthetic aminoglycoside antibiotics. Dibekacin (1975), amikacin (1977), netilmicin (1985), isepamicin (1988), and arbekacin (1990), which are marketed as chemotherapeutic agents, were developed by deoxygenation of the 3′-hydroxyl group as the modification site for APH(3′), and by 1-*N*-acylation, in order to synthesize dibekacin and the others, respectively. However, novel resistant bacteria to these antibiotics emerged sooner or later, and again were shown to be dependent on new types of aminoglycoside-modifying enzymes. In this regard, arbekacin, which is approved as an anti-MRSA agent, has been characterized by a low-to-moderate level of resistance, low incidence, and AAC(6′)/APH(2″)-dependence, in terms of the emergence of arbekacin-resistance in MRSA strains. Further, unexpected novel properties of arbekacin have been revealed by simulative modification studies using AACs of amnoglycoside-producing strains of *Streptomyces* (i.e., that the monoacetylated derivatives of arbekacin have substantial antibiotic activities). Therefore, we believe that arbekacin can be regarded as representing a new-generation aminoglycoside antibiotic, and that it provides a new direction, “double-stage activity” for developing new semisynthetic aminoglycoside antibiotics.

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